510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k042725

B. Purpose for Submission:

New submission

C. Measurand:

Opiates in hair

D. Type of Test:

Qualitative ELISA Immunoassay Test System, Home Brew

E. Applicant:

Quest Diagnostics, Inc.

F. Proprietary and Established Names:

Quest Diagnostics HairCheck-DT (Opiates)

G. Regulatory Information:

1. Regulation section:

   CFR 862.3650, Enzyme Immunoassay, Opiates and Opiates Metabolites

2. Classification:

   Class II

3. Product code:

   DJG

4. Panel:

   91 (Toxicology)

H. Intended Use:

1. Intended use(s):
   Refer to Indications for use.

2. Indication(s) for use:

   The Quest Diagnostics Hair Check-DT (Opiates) is a test system that utilizes the IDS One-Step ELISA Opiates Kit for the qualitative detection of opiates at or above 500 pg/mg in head hair samples from chronic heroin users. This test system has not been evaluated for use with other user populations or with hair specimens other than the head. It is an in vitro diagnostic device intended exclusively for in-house professional use only and is not intended for sale to anyone.

   The QUEST DIAGNOSTICS HairCheck-DT (Opiates) provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed result. Gas Chromatograph - Mass Spectrometry operating in the selected ion monitoring (SIM) mode or GC/MS/MS in selected reaction mode (SRM) is the preferred method with deuterated internal standards. Other chemical confirmation methods are available. Clinical consideration and professional judgment should be applied to any drug of abuse test result, particularly when preliminary positive results are obtained.

   Limitations: Evaluation of this assay was limited to head hair samples from a population of chronic heroin users. Interpretation of results must take into account that drug concentrations detected in hair from a single individual can vary extensively depending on the site of collection. Positive screening results only indicate the presumptive presence of opiates (codeine, morphine and/or 6-monoacetylmorphine), and require additional analysis by GC/MS or GC//MS/MS to confirm the result. A negative screening result does not necessarily rule out the possibility of opiate use, i.e., time of collection, frequency of use, mode of ingestion, dosage used, hair types and other factors may influence results.

   It is not possible to document all possible effects due to treatments such as bleaching, straightening and dying.

   There is a possibility that other substances and/or factors not evaluated in the interference studies may interfere with the test and cause false results that cannot be confirmed by mass spectrometry, e.g. technical or procedural errors.

3. Special conditions for use statement(s):

   The assay is for Prescription In-House Use.

4. Special instrument requirements:
   The device is for use with an automated microplate reader capable of measuring at 450 and 630 nm.

For confirmation testing, the sponsor uses an Agilent 5973N GC/MS in selected reaction monitoring (SRM) mode using deuterated internal standards.

I. Device Description:

The test obtaining clearance consists of two parts; a **pre-analytical** hair treatment procedure (to convert the solid matrix of hair to a measurable liquid matrix) and the **screening assay.** The screening assay, International Diagnostic Systems (IDS) Corporation One-Step ELISA (Enzyme-Linked ImmunoSorbent Assay) Opiates Kit, is purchased by Quest.

The screening portion of the test system consists of micro strip plates coated with rabbit anti-morphine polyclonal antibody, enzyme conjugate (horseradish peroxidase conjugated to morphine), substrate (containing tetramethylbenzidine), a proprietary diluent, and wash solution.

In-house prepared calibrators and controls are utilized. These are prepared solutions of morphine added to negative matrix tubes.

As the IDS kit is a component of the test system, the sponsor was asked to describe what specifications are provided to the component manufacturer, and what testing is performed when the kit is received to validate its performance. Details of the procedures used are described in Quest's 6/8/05 response. IDS performs a drift and precision procedure on selected plates, as well as visual inspection of all plates. Quest performs a linearity check on selected plates when they are received.

The procedures and reagents are briefly described in the "Test Principle" section, below. Trade secret information was not provided to FDA. Specific details regarding procedures and reagents provided by the sponsor have not been reproduced here because the product is not intended for sale to others.

The sponsor indicates there are no human source materials in their product, except for hair. Hair is not known to be a biological risk.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Dade Behring (Syva) EMIT II Opiates Assay

2. Predicate 510(k) number(s):

   k011289

3. Comparison with predicate:

   Both devices are qualitative assays for the detection of opiates. Both are immunoassays.

Differences		
Item	**Device**	**Predicate**
Method of measurement	Microplate reader	Spectrophotometer
Matrix	Head hair	Urine
Cutoff concentration	500 pg opiates/mg hair	2000 ng morphine/mL urine
Test Principle	ELISA	Competitive EIA

K. Standard/Guidance Document Referenced (if applicable):

No standards are referenced.

L. Test Principle:

Pre-Analytical:
The test utilizes a 3.9 cm sample of head hair. Approximately 120 strands taken from 2-3 different sites, and is cut as close as possible to the scalp, preferably from the back of the head at the crown. This amount of hair should weigh approximately 100–120 mg. In the laboratory the sample is cut from the root end, then cut into smaller lengths and mixed to ensure homogeneity.

Specimens are prepared by weighing out twenty milligram aliquots of the hair. In preparation for the screening test, an aliquot is washed with methanol for a brief period of time, then the methanol is discarded. This pre-wash is intended to rid the sample of external contamination. Methanol is added to the hair and it is heated for two hours. The methanol mixture is then transferred to a new tube and evaporated under nitrogen. The tubes are reconstituted with 0.6 mL of phosphate buffer prior to testing.

To minimize hair matrix effects calibrator and control stock solutions are added to a negative matrix tube prior to analysis. To prepare these tubes 10 grams of hair from non drug-users is weighed out and methanol is added. After soaking for a period of time the methanol is discarded. One liter of methanol is added to the methanol-washed hair and heated for 2 hours, then filtered. The collected methanol is diluted with methanol to 1 liter. One mL aliquots are pipetted into tubes and evaporated to dryness. Prior to analysis, 100 µL of prepared stock solutions of calibrator and control are pipetted into the negative hair matrix tubes, and 1.9 mL of phosphate buffer is added.

Screening Assay:
Unknown samples, calibrators, and controls, as described above, are assayed using the IDS Opiates Kit. The kit is a solid-phase micro-titer plate immunoassay where labeled and unlabeled opiates bind to antibody. The two bind in proportion to their concentration.

Each sample is added to a well, followed by the enzyme conjugate. During this phase of

incubation the enzyme-labeled drug conjugate competes with drug in the sample for a limited number of binding sites on the antibody-coated microwells. A wash solution is then applied to remove any unbound materials. Enzyme substrate solution containing a chromagen is then added for the final color development process. The reaction is stopped with an acid and the absorbance is read using a plate reader at 450 nm. A background reading is also taken at 630 nm. Color intensity is inversely proportional to the amount of analyte present in the sample.

Interpretation of Screening Results:
Negative: Samples with an absorbance value higher than the Cutoff Calibrator are interpreted as negative. Either the sample does not contain opiates or opiates are present in concentrations below the cutoff level for the assay.

Presumptive Positive: Samples with an absorbance value equal to or lower than the Cutoff Calibrator are presumptively positive.

Other structurally similar compounds can produce positive results. Compounds that are not structurally similar to opiates have not been observed to produce positive results, however false positive screening results may occur because of non-specific binding or other technical problems.

Confirmatory Testing:

Negative hair matrix tubes are used in the confirmatory process. As in the screening procedure, control and calibrator solutions are added to the tubes prior to analysis. Negative hair matrix tubes are prepared in a similar manner to those prepared for the screening assay.

Confirmation testing is performed utilizing another aliquot from the original hair specimen. A 20 mg sample of each donor hair sample is washed four times prior to analysis. The first wash is performed with hexane. This wash is saved and analyzed along with the donor sample. (The concentration of drug in the hexane wash is multiplied by ten, then subtracted from the GC/MS result prior to applying the positive reporting criteria. This step is performed to mitigate the risk of external drug contamination.) The hexane wash is followed by 3 additional methanol washes. Each methanol wash is discarded.

Methanol is then added to the sample and it is heated for 2 hours. An acid/methanol mixture is then added, then the liquid is transferred to another tube. It is evaporated to dryness, and reconstituted with phosphate buffer.

A solid phase extraction is then performed on each standard, control, and unknown specimen, followed by GC/MS analysis. The hexane wash correction procedure is performed prior to determining the final test result.

Interpretation of Confirmatory Testing Results: Samples are considered positive for opiates use if morphine or codeine or 6-MAM are present at or above 500 pg/mg of hair.

For the run to be accepted, at least one control must be within 30% of the target value. (If only

one control passes, results are treated as qualitative results, rather than quantitative results.)

According to the sponsor, LOD's for morphine, codeine and 6-MAM on their GC/MS system are 100, 200 and 100 pg/mg, respectively.

M. Performance Characteristics (if/when applicable):

Two terms are utilized when describing study results.

- Normalized data is where the absorbance reading of the cutoff calibrator is considered. Formula for calculating the normalized absorbance value of unknown samples is: (1/absorbance value of cutoff calibrator) x absorbance value of unknown
- Non-normalized data refers to the raw absorbance readings observed in the run.

1. Analytical performance:

a. Precision/Reproducibility:

A variety of specimen types were evaluated in precision studies, i.e., morphine solutions spiked into negative matrix tubes, pooled extracts of hair samples, and replicates of individual hair samples.

Within-run and Between-run Precision Using Spiked Samples

Five solutions were prepared at 0, 0.5X, 1X, 1.5X and 2X of cutoff. These solutions were prepared by spiking test tubes containing negative hair matrix with the appropriate concentration of morphine to provide the percentages of cutoffs listed above. On day one, 15 replicates of each solution were pipetted into individual wells on a Microtiter plate and then analyzed by the ELISA screening method listed above. The data from day 1 was used to establish the Within-run precision for the ELISA screening method.

From these same solutions, 15 replicates were again analyzed in individual wells on days 2 and 3. Data from all 3 days was used to determine the Between-run precision, i.e., 45 replicates.

One experienced laboratory employee performed this study at Quest.

A total of 225 samples were assayed:

- 15X3 - 0.0 pg/mg Morphine
- 15X3 - 250 pg/mg Morphine
- 15X3 - 500 pg/mg Morphine
- 15X3 – 750 pg/mg Morphine
- 15X3 - 1000 pg/mg Morphine.

Calculations: Within-run precision was determined from Day 1 data for each concentration by calculating the mean, standard deviation and coefficient of variation for 15 samples. Between-run precision was determined by calculating the mean, standard deviation, and coefficient of variation for each concentration, 45 samples each, over three days. Precision is expressed as the percent coefficient of variation (%CV), where the standard deviation divided by the mean times 100% is equal to the percent coefficient of variation.

Within-Run Precision Study using Spiked Samples (non-normalized data)

Opiates Spiked Sample	Negative	50%	100%	150%	200%
Mean	2.217	1.257	0.400	0.253	0.211
95% CI Upper Limit	2.267	1.297	0.413	0.26	0.218
95% CI Lower Limit	2.167	1.218	0.386	0.246	0.214
S.D.	0.091	0.071	0.025	0.013	0.013
95% CI Upper Limit	0.575	0.446	0.157	0.081	0.080
95% CI Lower Limit	0.048	0.037	0.013	0.007	0.007
CV%	4.1%	5.6%	6.2%	5.1%	6.0%
95% CI Upper Limit	25.9%	35.5%	39.3%	32.1%	37.7%
95% CI Lower Limit	2.1%	2.9%	3.3%	2.7%	3.1%

Between-Run Precision using Spiked Samples (non-normalized data)

Opiates Spiked Sample	Negative	50%	100%	150%	200%
Mean	2.008	1.148	0.354	0.220	0.181
95% CI Upper Limit	2.041	1.169	0.361	0.224	0.186
95% CI Lower Limit	1.976	1.126	0.347	0.215	0.177
S.D.	0.059	0.039	0.013	0.008	0.008
95% CI Upper Limit	0.371	0.244	0.082	0.052	0.053
95% CI Lower Limit	0.031	0.020	0.007	0.004	0.004
CV%	2.9%	3.4%	3.7%	3.8%	4.7%
95% CI Upper Limit	18.5%	21.3%	23.1%	23.7%	29.4%
95% CI Lower Limit	1.5%	1.8%	1.9%	2.0%	2.4%

Within-run and Between-Run Precision using pooled extracts

Within-run precision was determined by analyzing fifteen replicate samples of pooled sample extracts. Results were similar to those of prepared solutions, above.

Within-Run Precision using individual samples

Studies were done to characterize precision when replicate measurements of single hair samples were analyzed. Four hair specimens previously found to render absorbance readings close to the absorbance reading of the cutoff calibrator were analyzed. Each hair specimen was divided into 3 three aliquots of 20 mg each. Each 20 mg aliquot was taken through the entire ELISA screening process and measured in one run. The following table depicts the absorbance readings (not normalized) of the analysis.

Within-Run Precision of Opiates using individual hair (non-normalized data)

Specimen #	**1**	**2**	**3**	**4**
	0.105	1.377	0.058	0.061
	0.094	1.289	0.062	0.047
	0.080	1.189	0.048	0.065
Mean	**0.093**	**1.285**	**0.056**	**0.058**
95% CI				
Upper Limit	0.124	1.519	0.074	0.081
Lower Limit	0.062	1.051	0.038	0.034
Std Dev.	**0.013**	**0.094**	**0.007**	**0.009**
95% CI*				
Upper Limit	0.079	0.591	0.045	0.059
Lower Limit	0.007	0.049	0.004	0.005
%CV	**14.0%**	**7.3%**	**12.9%**	**16.4%**
95% CI*				
Upper Limit	87.8%	46.0%	80.9%	103.0%
Lower Limit	7.3%	3.8%	6.7%	8.5%

Calibrator absorbance in this run was 0.577 with low and high control of 1.493 and 0.339 respectively. The absorbance value of the negative control was 2.004

b. Linearity/assay reportable range:
Not applicable. This is a qualitative assay. Representative absorbances are observable, however, in the precision section, above.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Commercially purchased materials consisting of morphine in methanol are used to prepare a working solution. Working solutions are then used to prepare calibrator and control solutions. (Calibrators and controls are prepared in a similar manner, however, they are made from different reference materials, each provided with a Certificate of Analysis.)

Assigned values of the gravimetrically prepared calibrator and control stock solutions

are verified by GC/MS analysis each time a new batch is prepared. The calibrator must fall within 20% of the targeted concentration. The sponsor indicates they have data on file to support the one year expiration date for these solutions.

At the time of analysis, the prepared calibrator and control stock solutions are pipetted into a negative matrix tube, and diluted with phosphate buffer. The final concentrations are as follows:

- Positive Calibrator containing 500 pg/mg hair of opiates
- Negative Blank Calibrator (negative matrix tube containing 0.0 pg/mg hair of opiates)
- Low Control containing 400 pg/mg hair of opiates
- High Control containing 600 pg/mg hair of opiates

Users are instructed to follow federal, state, and local regulatory guidelines regarding quality control procedures.

d. Detection limit:

The limit of detection (in pg/mg) was determined by calculating the mean negative calibrator absorbance (A_0) minus two times the SD (LOD= A_0-2SD).

The calculation of sensitivity was determined in hair matrix samples by calculating the mean absorbance value of each set of 18 zero calibrators (blanks) and adding two standard deviations for the corresponding group. To convert the value from absorbance units to pg/mg concentration, a regression line was constructed using the mean values of the zero standard, 225 pg/mg morphine standard, 300 pg/mg morphine standard, and 375 pg/mg morphine standard. Using the equation of the regression line, the absorbance value of the mean zero calibrator minus two standard deviations was converted to pg/mg of morphine.

One experience laboratory employee performed this study in one day.
The study took place at the Quest laboratory.

LOD Study Results

	0 pg/mg in buffer + matrix (Absorbance)
Mean (0 pg/mg)	1.920
Std Dev	**0.090**
Std Dev X 2	**0.180**
Mean-2 Std Deviations	1.740
Mean (225 pg/mg)	0.676
Mean (300 pg/mg)	0.572
Mean (375 pg/mg)	0.428
R Square	**0.943**

Slope	**-0.004**
Intercept	**1.827**
(y-b)/m	**(1.827-1.920)/-.004**
LOD (pg/mg)	21

e. Analytical specificity:

Cross-Reactivity with structurally related compounds:

To determine cross-reactivity each compound was spiked into 46 mm phosphate buffer containing negative hair matrix.

Serial dilutions of each compound were prepared and analyzed. Resulting absorbance readings were plotted against the prepared concentration. The concentration of each compound that generated the same absorbance reading as the cutoff calibrator was extrapolated from the graph. The concentration of morphine in the cutoff calibrator was divided by the extrapolated concentration of the structurally similar compound and then multiplied by 100. (For example if it took 1200 pg/mg of a structurally similar compound to equal the absorbance value of 500 pg/mg of morphine then the cross reactivity would be 500/1200 x 100% = 50%.)

Percent Cross-reactivity of structurally related compounds

Compound	Percent Cross-Reactivity	Amount (pg/mg) of Morphine Analog equivalent to produce a positive result at the cut-off of 500 pg/mg
Ethylmorphine	555.6	90
Codeine	285.7	175
6-Acetylcodeine	133.3	375
Heroin	125	400
Dihydrocodeine	111.1	450
Morphine	100	500
6-Monoacetylmorphine	100	500
Morphine-3-beta-glucuronide	100	500
Thebain	83.3	600
Morphine-6-beta-glucuronide	76.9	650
Dihydromorphine	71.4	700
Hydrocodone	33.3	1502
Hydromorphone	33.3	1502
Nalorphine	15.4	3247

Compound	Percent Cross-Reactivity	Amount (pg/mg) of Morphine Analog equivalent to produce a positive result at the cut-off of 500 pg/mg
Levorphanol	14.3	3497
Norcodeine	5	10000
Oxycodone	1.1	45455
Normorphine	0.7	71429
Diprenorphine	0.6	83333
Oxymorphone	0.5	100000
Apomorphine	0.5	100000

Other compounds tested demonstrated less than 0.5% cross-reactivity.

Cross-Reactivity with structurally unrelated compounds

Several (160) structurally unrelated compounds were added to 46 mm phosphate buffer to a concentration of 10,000 ng/mL then added to negative hair matrix tubes (equivalent to 300,000 pg/mg). Samples were analyzed along with replicates of blank negative hair matrix tubes. The mean absorbance readings from the samples were within 5% of the mean absorbance readings of the blank negative hair matrix tubes.

Effect of Interfering Compounds

The same 160 structurally unrelated compounds were also tested for possible positive and negative interference with the Opiate ELISA assay. Two sets of negative hair matrix were prepared by adding morphine to achieve concentrations of at 333, 500 and 667 pg/mg hair. The second set of tubes was additionally spiked with the 160 structurally unrelated compounds to a concentration of 300,000 pg/mg hair. Absorbance readings of the tubes spiked with the structurally unrelated compound were within 5% of the absorbance readings of the negative hair matrix tube without the compound added.

There is a possibility that other substances and/or factors not listed above may interfere with the test and cause false results e.g., technical or procedural errors.

ADDITIONAL ANALYTICAL STUDIES:

Recovery studies/ Effectiveness of Screening Assay Extraction Method

The screening assay employs a process to extract drug from a hair sample, i.e., a 2-hour methanolic extraction of hair at 70^{o}C. A study was done to demonstrate the effectiveness of this procedure.

In order to evaluate the effectiveness of the methanolic extraction procedure a definition of 100% recovery was established. According to the sponsor, treatment of hair with a strong base (NaOH) converts hair to liquid, which can be extracted by a solid phase extraction

technique for analysis. The strong base also converts a significant amount of 6-Acetylmorphine to morphine. Quest defines total recovery in this study as the sum of the codeine, morphine and 6-acetylmorphine as quantitated by GC/MS.

Two sample types were evaluated:

1) Hair spiked with morphine and 6-acetylmorphine:

   Approximately 1 gram of drug-free hair was soaked in an aqueous solution containing 1000 ng/mL of morphine and 6-acetylmorphine for 24 hours. The hair was removed from the solution and washed with "Aloe Rid" shampoo once, water 5 times and allowed to air dry overnight at room temperature.

2) Hair samples which previously confirmed positive for morphine, codeine and/or 6-acetylmorphine.

Samples were aliquoted in duplicate; one aliquot was taken through the screening extraction and assay (described in Test Principle Section, above) and the other was taken through the 100% recovery extraction.
To perform extraction recovery determinations of each opiate compound, the screening extraction procedure was performed up to the point of evaporating the methanolic extract. At that point, internal standard was added and the solid phase extraction procedure used during confirmation testing was performed, followed by GC/MS analysis.

The method representing 100% recovery was accomplished by adding 0.5 mL of 1.0 N NaOH to 20 mg of hair and incubating at 70^{o}C for 30 minutes. The sample is totally liquefied and the entire drug originally bound to the hair is now dissolved in the base solution. After base hydrolysis of the hair, the internal standard is added and the confirmation solid phase extraction procedure was performed.

The GC/MS results of the screening extraction were compared to the results of the confirmation extraction to determine the relative recovery of the drug using the 2-hour methanol incubation:

GC/MS Results and Recovery

	Methanol				Base Hydrolysis				% Recovery
Sample	COD	MOR	6-MAM	**TOTAL**	COD	MOR	6-MAM	**TOTAL**	**TOTAL**
1	168	3680	21	3869	173	4022	36	4231	**91.4%**
2	0	96	7	103	0	65	69	134	**76.9%**
3	102	25	0	127	164	157	67	388	**32.7%**
4	1254	33	0	1287	2114	40	0	2154	**59.7%**
5	211	13	0	224	213	88	0	301	**74.4%**
Spiked #1	0	2363	6487	8850	0	9303	65	9368	**94.5%**
Spiked #2	0	1980	5434	7414	0	8269	102	8371	**88.6%**
Spiked #3	0	1726	4834	6560	0	10761	78	10839	**60.5%**
					Average Recovery of Positive Donor Samples				**67.0%**
					Average Recovery of Spiked Samples				**81.2%**
					Average Recovery of All Samples				**72.3%**

Hair Treatment Effects on Positive Hair Samples:

The effects of various hair treatments (i.e. bleaching, dyeing, shampooing) on the screening and confirmatory assay was examined. Forty-eight previously screened and confirmed opiate positive hair specimens were randomly assigned into one of three groups (16 in each group). Each group was subjected to one of the three treatments. Absorbance readings and GC/MS measurements were compared before and after treatment. Each of the three opiate compounds were examined separately during GC/MS analysis. Results appear below.

**Note: An increase in concentration correlates to a decrease in Absorbance and a decrease in concentration correlates with an increase in Absorbance.

Effects Observed in the Bleaching Study (Normalized data, n= 16)

		ELISA SCREENING DATA			
	Avg Abs/ Range of Abs *	# of samples that remained positive	Avg/ Range of Abs of all that had a decrease in Abs **	# of samples that became negative	Avg/ Range of Abs of all that had an increase in Abs **
Untreated	0.135 (0.042 – 0.358)				
Treated	3.859 (2.721 – 4.247)	0	0	16	3.859 (2.721 – 4.247)
		GC/MS CONFIRMATION DATA			
	Avg / Range of sample concentrations (pg/mg)	# of samples that decreased in concentration	Avg/ Range of decrease in concentration	# of samples that increased in concentration	Avg/ Range of increase in concentration
Untreated					
Codeine	1443 (297– 4432), n=7				
Morphine	899 (159-4189), n=14				
6 MAM	4369 (1225-14116), n=14				
Treated					
Codeine	143 (0-585)	7	143 (0-585)	0	-
Morphine	None Detected	14	None Detected	0	-
6 MAM	95(0-370)	14	95 (0-370)	0	-

Effects Observed in the Dyeing Study (Normalized data, n= 16)

		ELISA SCREENING DATA			
	Avg Abs/ Range of Abs*	# of samples that remained positive	Avg/ Range of Abs of all that had a decrease in Abs **	# of samples that became negative	Avg/ Range of Abs of all that had an increase in Abs **
Untreated	0.178 (0.025-0.410)				
Treated	2.300 (0.139-3.514)	2	0	14	2.300 (0.139 –3.514)
		GC/MS CONFIRMATION DATA			
	Avg / Range of sample concentrations (pg/mg)	# of samples that decreased in concentration	Avg/ Range of decrease in concentration	# of samples that increased in concentration	Avg/ Range of increase in concentration
Untreated					
Codeine	586 (394-809), n=4				
Morphine	815 (203-3894), n=13				
6 MAM	6911 (710-33032), n=14				
Treated					
Codeine	None detected	4	None detected	0	-
Morphine	521 (0-4610)	11	141 (0-1045)	2	2613 (616-4610)
6 MAM	772 (0-6420)	14	772 (0-6420)	0	-

Effects Observed in the Shampoo Study (Normalized data, n= 16)

		ELISA SCREENING DATA			
	Avg Abs/ Range of Abs*	# of samples that remained positive	Avg/ Range of Abs of all that had a decrease in Abs **	# of samples that became negative	Avg/ Range of Abs of all that had an increase in Abs **
Untreated	0.220 (0.062-0.522)				
Treated	0.450 (0.095-2.558)	14	0.139 (0.139-0.139)	2	0.471 (0.095-2.558)
		GC/MS CONFIRMATION DATA			
	Avg / Range of sample concentrations (pg/mg)	# of samples that decreased in concentration	Avg/ Range of decrease in concentration	# of samples that increased in concentration	Avg/ Range of increase in concentration
Untreated					
Codeine	896 (176-2788), n=5				
Morphine	532 (124-1684), n=11				
6 MAM	3608 (401-15681), n=14				
Treated					
Codeine	951 (0-2938)	2	81 (0-162)	3	1531 (216-2938)
Morphine	360 (0-1007)	8	393 (0-1007)	3	270 (149-375)
6 MAM	3170 (486-15443)	9	3258 (486-15443)	5	3011 (838-7821)

Hair treatment Affects on Negative Hair:

In a separate study 52 previously screened negative specimens were randomly assigned to the same treatment groups. All 52 specimens remained negative after treatment. The percent difference between the mean normalized absorbance values of the treated and untreated groups was –8.65%, -9.82% and 0.69% for bleaching, dyeing and shampooing respectively.

Bleaching and dyeing had the greatest effect. Screening absorbance readings became more negative for the positive hair samples, and slightly more negative for the negative hair samples. (The decrease in absorbance reading is equal to an apparent increase in concentration.)

Effectiveness of the Wash Procedure (Contamination Studies):

Two studies were done to determine whether confirmatory testing procedures (including the hexane wash correction) are able to distinguish between true analytically positive samples and those that have been externally exposed to drug. The focus of the studies was to demonstrate that the hexane wash correction procedure mitigates the risk of false positive results while not altering true analytical positive results.

1. The first study involved exposing drug-free hair to opiate compounds, performing confirmation testing on the samples and observing the final test result.
2. The second study involved performing confirmation testing on known positive samples and observing whether the hexane wash correction altered the final result.

STUDY # 1

Five hair specimens that had previously screened negative for opiates were selected in order to represent five hair types.

Hair Types:

Category	Hair Color	Hair Texture
A	Black	Straight
B	Black	Curly
C	Brown	Thin
D	Brown	Thick
E	Blonde	All types

One aliquot of each hair type was exposed to morphine and codeine (in separate experiments) according to the first three exposure modes listed below. Another aliquot of each hair type was exposed to heroin smoke. A twenty mg aliquot of all hair samples was then analyzed by GC/MS. Results are presented for each exposure mode, and according to the drug and category of hair types.

Exposure modes

#	Type of Exposure	How performed
1	Dry Contact	Ten (10) mg of morphine sulfate and ten (10) mg of codeine sulfate were weighed and combined with 10 grams of dextrose with maltodextrin, aspartame (Equal®). The powdered mixture was grinded to a fine powder and mixed thoroughly. Exactly 150 mg of powder was added to 150 mg of each hair type in individual plastic bags and the bags closed with a ziplock® seal. Bags were thoroughly shaken, manipulated and rubbed to ensure direct contact between the powder and hair. Hair specimens remained in the plastic bags for one hour.
2	Dry Contact plus water	After treatment by exposure mode #1, above, twenty mg aliquots of hair were weighed out and 2.0 mL of deionized water was added and then quickly removed to simulate rinsing the hair, as in a shower. An additional 2.0 mL of deionized water was then added and the tube was allowed to stand for 30 minutes at ambient temperature. The water was then removed and the hair dried overnight.
3	Dry Contact plus Saline	After treatment by exposure mode #1, above, twenty mg aliquots of hair were weighed out and 2.0 mL of 0.9% saline solution was added. The tube stood for 30 minutes at ambient temperature. Saline solution was removed and the hair dried overnight.
4	Smoke	One hundred mg of each hair type was exposed to heroin smoke by pyrolyzing 1.0 mg of diacetylmorphine (Heroin) in a glass device. The glass device was connected to carrier gas on one end and then connected to a tube containing the hair on the other end. The powder was burned and once smoke was produced, the gas was turned on which carried the smoke to the tubes containing the hair specimen. Smoke was held in the test tube for 5 minutes.

Results from the study are presented in the tables below.

Morphine Exposure:

Exposure mode 1

	Morphine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	(D – A)
A	10501	28	105010	-104982
B	16409	65	164090	-164025
C	2640	73	26400	-26327
D	22762	279	227620	-227341
E	13587	90	135870	-135780

Codeine Exposure:

Exposure mode 1

	Codeine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	(D – A)
A	2690	148	26900	-26752
B	3642	151	36420	-36269
C	3024	209	30240	-30031
D	209797	1452	2097970	-2096518
E	4176	157	41760	-41603

Morphine Exposure:

Exposure mode 2

	Morphine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	D – A)
A	239	1546	2390	-844
B	2539	5543	25390	-19847
C	350	342	3500	-3158
D	3248	9203	32480	-23277
E	819	944	8190	-7246

Codeine Exposure:

Exposure mode 2

	Codeine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	(D – A)
A	89	252	890	-638
B	124	894	1240	-346
C	83	342	830	-488
D	581	2802	5810	-3008
E	363	348	3630	-3282

Morphine Exposure:

Exposure mode 3

	Morphine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	D – A)
A	428	1311	4280	-2969
B	1978	4449	19780	-15331
C	1885	832	18850	-18018
D	829	3360	8290	-4930
E	3398	2612	33980	-31368

Codeine Exposure:

Exposure mode 3

	Codeine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	(D – A)
A	273	300	2730	-2430
B	70	409	700	-291
C	2250	769	22500	-21731
D	1174	1147	11740	-10593
E	511	464	5110	-4646

Heroin Exposure:

Exposure mode 4

	Morphine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	D – A)
A	6056	2037	30280	-28243
B	9534	244	47670	-47426
B1	23277	735	116385	-115650
C	126	5	630	-625
D	70	12	350	-338
E	1054	132	5270	-5138

Heroin Exposure:

Exposure mode 4

	6 MAM (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	D – A)
A	35850	4047	358500	-354453
B	19432	1320	194320	-193000
B1	22902	14454	229020	-214566
C	516	13	5160	-5147
D	408	101	4080	-3979
E	181	71	1810	-1739

STUDY #2

Nine clinically positive hair samples were selected for this study. All hair samples:

-were previously screened and confirmed positive
-came from subjects who admitted opiate or heroin use
-were accompanied by a urine specimen that screened positive

Confirmation testing procedures were performed on the 9 samples, i.e., 4 washes, extraction SEP, GG/MS, and hexane wash correction. Results are presented below:

CLINICAL POSITIVE SAMPLES

	Morphine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	(E) = D minus A (pg/mg) Hair
#30	13	5998	130	**5868**
#35	11	6184	110	**6074**
#3	0	358	0	**358**
#13	0	1762	0	**1762**
#18	0	329	0	**329**
#45	0	484	0	**484**
#47	0	608	0	**608**
#70	0	322	0	**322**
#71	0	240	0	**240**

	Codeine (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	((E) = D - A (pg/mg) Hair
#30	0	495	0	**495**
#35	0	891	0	**891**
#3	0	40	0	**40**
#13	0	275	0	**275**
#18	0	52	0	**52**
#45	0	265	0	**265**
#47	0	0	0	**0**
#70	0	0	0	**0**
#71	0	41	0	41

	6 MAM (pg/mg)			
	(A) Hexane Wash	(B) Final Extract	(D) x10 of Hexane Wash	(E) = B minus D
#30	48	22401	480	**21921**
#35	30	9458	300	**9158**
#3	0	5300	0	**5300**
#13	203	25847	2030	**23817**
#18	0	4031	0	**4031**
#45	0	3995	0	**3995**
#47	134	9336	1340	**7996**
#70	0	3692	0	**3692**
#71	0	2469	0	2469

Summary of Study Results: Results from the analytically negative samples remained negative after being exposed to the three opiate compounds under the described conditions. All clinically positive samples remained positive.

Stability Study:

Eleven samples previously screened positive for Opiates and analyzed by GC/MS for morphine, codeine and 6-monoacetylmorphine (6-MAM) were used in this

study. Samples were stored in a climate controlled space, then analyzed a second time approximately 3 years later.

The following table illustrates the mean concentration of morphine, codeine and 6-MAM in the samples. Based on this data there is an overall trend of drug loss.

Results From Stability Study on eleven Samples

Study Observation	Morphine	Codeine	6-MAM
Average Concentration, pg/mg hair, Baseline	505	137	2045
Mean Change in %	- 29 %	- 18 %	-31%
Range in concentration, pg/mg hair	0-2055	0-786	0-12172
% Maximum Decrease	-100%	-100%	-66%
% Maximum Increase	+81%	+100%	+33%
Number that increased in concentration	3	3	1
Number that decreased in concentration	5	5	9
Number that did not contain the specific drug at the beginning or ending of the study.	3	3	1

Drugs rendering a 0 pg/mg hair result at initial testing were excluded from calculations involving a percent change.

Three of the eleven specimens analyzed changed from detectable to undetectable concentrations of both morphine and codeine. The original concentration of codeine in these three specimens was 116, 122 and 122 pg/mg. The original concentration of morphine in these three specimens was 424, 539 and 677 pg/mg.

10 of the 11 original specimens contained of 6-MAM. All ten contained measurable amounts of 6-MAM three years later. All eleven of the original specimens contained measurable amounts of one or more of the drugs three years later.

f. Assay cut-off:

The Substance Abuse and Mental Health Services Administration has not yet recognized hair testing in the Federal Workplace Drug Testing program. Preliminary recommendations, however, suggest the use of a 200 pg/mg cut-off level for opiates as the initial screening level. Confirmatory recommendations are

for there to be 200 pg/mg of either morphine, codeine, or 6-acetylmorphine to be reported as positive.
A screening cutoff of 500 pg/mg of opiates is used by Quest Diagnostics. The positive agreement studies appear to support that this is an effective cutoff for the claimed user population, i.e., chronic users.

Characterization of how the device performs analytically around the claimed cutoff concentration appears in the precision section, above.

2. Comparison studies:

a. Method comparison study: Agreement Studies

Clinical performance was evaluated with two studies, one involving chronic heroin users and one involving self-reported non-drug users. (Hexane wash correction procedures were not used at the time of these studies.)

Positive Agreement Studies

The study enrolled 105 subjects known to be chronic Heroin users and/or had a positive urine test for Opiates. Almost all participants reported last using heroin on a daily basis. Participants also indicated they had been using heroin for anywhere from 1 to 40 years. Each subject provided a urine and head hair sample.

Of the 105 volunteer subjects 88 were Caucasian, 16 Hispanic and one (1) did not note their race. The subjects ranged in ages from 21 to 70 years old. Of the 105 hair samples: 10 were light brown, 52 were medium brown, 33 were dark brown, 4 were gray, 3 were black and 3 were blonde. The curvature ranged from 68 straight and 34 curly. There was not sufficient hair to evaluate 3 of these samples as to curvature.

One hundred and five (105) were positive for Opiates in their urine using EMIT (300 ng/mL cutoff). Urine specimens were not confirmed by GC/MS. Ninety-seven (97) of the hair samples screened positive using ELISA (500 pg/mg hair cutoff). Ninety (90) of the hair samples confirmed positive for combinations of codeine, morphine or 6 monoacetyl morphine above the 500 pg/mg GC/MS cut-off. A positive was determined to be a value greater than 500 pg/mg hair for any of the three opiate analytes. The following table describes the findings.

Positive Agreement Study Results

Subjects	Urine (EMIT) Results	Hair ELISA Results	Hair GC/MS Results	History on Survey
83	+	+	+	+
7	+	+	+	-
1	+	+	-	-
5	+	+	-	+
1	+	+	QNS	+
8	+	-	-*	+

* These eight specimens that screened positive in urine but screened negative in hair. These specimens were analyzed by GC/MS and were all found to contain at least two opiate compounds; all specimens contained Morphine (32-288 pg/mg hair) and 6MAM (40-225 pg/mg hair), three specimens contained Codeine (22- 51 pg/mg hair). In all cases, the concentrations were below the identified screening and confirmatory cutoff concentrations.

Positive Agreement Study ELISA absorbance value information:
Average Absorbance of the cutoff calibrator was 0.711
Normalized Absorbance range of 97 screened positive samples was 0.029 to 0.581
Average normalized absorbance value of all 105 enrollees is 0.305
QNS – specimen insufficient for GC/MS analysis

Negative Agreement Study

Eighty-two (82) individuals who self-reported that they were non-drug users were enrolled in the study. Subjects provided urine and a hair sample.

Of the eighty-two (82) samples only thirty had race recorded. Twenty-three were Caucasian, 4 were African-American and 3 were Hispanic. No ages were collected on any of the volunteers. Of the eighty-two hair specimens 14 were black, 23 were dark brown, 20 were medium brown, 11 were light brown, 12 were blond and 2 were red. The curvature of the samples were estimated to be 45 straight, 32 curly, and 5 kinky.

All eighty-two (82) urine samples screened negative for Opiates using EMIT (300ng/mL cutoff). Urine samples were not confirmed. All 83 hair samples screened negative and contained no measurable amounts of Opiates when analyzed by GC/MS.

Negative Agreement Study Results

Number of Subjects	Urine Results Screen	Hair Results Screen	Hair GC/MS Result
82	-	-	-

Negative Agreement Study ELISA absorbance value information:

Normalized absorbance values for specimens in this negative agreement study ranged from 1.672 to 2.688 with a mean absorbance value of 2.213.

There were two hair runs included in the negative agreement study. The absorbance of the cutoff calibrator in the first run was 0.861. The range of absorbance values for the samples in that run was 1.478 to 2.075, with an average absorbance value of 1.875. The absorbance of the hair cutoff calibrator in the second run was 0.817. Samples in this run ranged from 1.366 to 2.196, with an average absorbance value of 1.825.

Normalized Absorbance Readings from the two study populations

The following table displays the normalized absorbance readings from the negative and positive agreement studies.

Normalized data from Clinical Agreement Studies

	Negative Agreement	Positive Agreement
Mean Absorbance	2.213	0.164
Lowest Absorbance	1.672	0.043
Highest Absorbance	2.688	0.866
Standard Deviation	0.188	0.154
Mean Absorbance ± 2SDs	2.213 ± 0.376	0.164 ± 0.308

b. Matrix comparison:
Not applicable. The assay is intended for only one sample matrix.

3. Clinical studies:

a. Clinical Sensitivity:

The clinical study conducted characterized the effectiveness of the Quest test system to identify chronic heroin users satisfying the inclusion criteria for the study. Performance in a general population of users is unknown.

To estimate confirmation rates of samples run in their lab that screened

positive, the sponsor analyzed 3 months of recent historical data. Of those samples approximately 37% of them confirmed positive. Some of the samples that failed to confirm contained drug, but the concentrations were below the positive reporting criteria.

*b. Clinical specificity**:***

Clinical specificity of this drug is estimated in the negative agreement studies. However, the sample size is not adequate to rule out the possibility of a positive screening assay when no drug is present.

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**
   Not applicable

5. Expected values/Reference range:
   Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.